S/N 09/202,634 <u>PATENT</u>

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:

SCHUBERT ET AL.

Examiner:

J. EINSMANN

Serial No.:

09/202,634

Group Art Unit:

1634

Filed:

MARCH 3, 1999

Docket No.:

9725.13USWO

Title:

OZONE-INDUCED GENE EXPRESSION IN PLANTS

MARKED UP VERSION SHOWING CHANGES MADE

IN THE SPECIFICATION

Pages 32 and 33 have been deleted.

The paragraph corresponding to the table at page 34 has been amended as follows:

| VstI promotor | Examined, | GUS-enzyme activity | | induction |
|---------------|---------------|----------------------------|---------------------------|-----------------|
| 5'-deleted | independent, | [pmol MU min ⁻¹ | mg ⁻¹ protein] | factor |
| to position | transgenic, | + ozone | - ozone | |
| | tobacco lines | | | |
| -1500 | 1F(1) | 735 ± 100 | 63 <u>+</u> 7 | 11.7 |
| -740 | 2(F1) | 388 <u>+</u> 59 | 30 <u>+</u> 5 | 12.9 |
| -550 | 3(F0) | 126 ± 13 | 12 ± 3 | 10.5 |
| | 2(F1) | 173 ± 25 | 15 ± 3 | 11.5 |
| -500 | 5(F0) | 148 ± 52 | 15 <u>+</u> 6 | 9.9 |
| -430 | 6(F0) | 141 <u>+</u> 38 | 14 <u>+</u> 4 | 10.0 |
| -280 | 6(F0) | 22 <u>+</u> 4 | 13 ± 3 | 1.7 |
| | 2(F1) | 30 ± 3 | 15 <u>+</u> 3 | [20] <u>2.0</u> |
| -140 | 2(F0) | 12 + 0.2 | 8 ± 3 | 1.5 |
| | 3(F1) | 24 ± 3 | 15 ± 3 | 1.6 |
| -40 | 3(F1) | 24 <u>+</u> 2 | 15 ± 3 | 1.6 |
| +70 | 1(F1) | 3.5 ± 1 | 3.5 <u>+</u> 1 | 1.0 |
| | | | | |

IN THE CLAIMS

Claims 5-7, 12-14, 16, 17, 22, 24-26, 29-32, and 34-37 have been cancelled without prejudice.

Claims 1-4, 8-11, 15, 18-21, 23, 27, 28, and 33 have been amended and new claims 38-43 have been added as follows:

1. (Amended) [Plant DNA] An isolated nucleic acid comprising of the sequence:

ACTTTTCGAG CCCCTTGAAC TGGAAATTAA TACATTTTCC ACTTGACTT

TGAAAAGGAG GCAATCCCAC GGGAGGGAAG CTGCTACCAA CCTTCGTAAT

GTTAATGAAA TCAAAGTCAC TCAATGTCCG AATTTCAAAC CTCANCAAC

CAATAGCCAA T (SEQ ID NO: 1),

as set forth in Claim 4.

- 2. (Amended) [DNA sequence, as set forth in] The isolated nucleic acid of Claim [1] 4, which originates from grapevine (Vitis vinifera).
- 3. (Amended) [DNA sequence, as set forth in] The isolated nucleic acid of Claim [1] 4 which is naturally contained in the stilbene-synthase gene Vst1 [and corresponds to] at base pairs -270 to -430.
- 4. (Amended) [DNA-sequence which in relation to the DNA-sequence, as set forth in Claim 1, has] An isolated nucleic acid

having a sequence identity of at least 40% [, especially of at least 60%,] to a nucleic acid having sequence:

ACTTTCGAG CCCCTTGAAC TGGAAATTAA TACATTTCC ACTTGACTT
TGAAAAGGAG GCAATCCCAC GGGAGGGAAG CTGCTACCAA CCTTCGTAAT
GTTAATGAAA TCAAAGTCAC TCAATGTCCG AATTTCAAAC CTCANCAAC
CAATAGCCAA T (SEQ ID NO: 1),

and which conveys an ozone-inducible gene expression, or

which is a derivative, or

an allelic variant of the [DNA-sequence set forth in Claim 1, and] isolated nucleic acid sequence:

ACTTTTCGAG CCCCTTGAAC TGGAAATTAA TACATTTTCC ACTTGACTT

TGAAAAGGAG GCAATCCCAC GGGAGGGAAG CTGCTACCAA CCTTCGTAAT

GTTAATGAAA TCAAAGTCAC TCAATGTCCG AATTTCAAAC CTCANCAAC

CAATAGCCAA T (SEQ ID NO: 1),

which differs from said sequence by naturally occurring or artificially introduced variations [, such as deletions, insertions, substitutions, additions, recombinations,] and which [is able to] conveys [an] ozone-inducible gene expression.

- 8. (Amended) [Chimeric nucleic molecules into which has been inserted a DNA-sequence,] A chimeric nucleic acid molecule comprising the sequence as set forth in Claim [1] 4 [, or at least a fragment thereof which can convey] or an ozone-inducible [gene expression] fragment thereof [, with the exception of nucleic acid molecules that comprise the Vst1 promoter region occurring naturally in the Vst1-promotor 3' of the sequence, set forth in Claim 1, as well as said sequence].
- 9. (Amended) [Chimeric nucleic molecules, as set forth in] The chimeric nucleic acid molecule of Claim 8 further comprising coding regions, [which render possible an] wherein the chimeric molecule is renders ozone-inducible expression of the coding regions in plants [contained in said molecules].
- 10. (Amended) [Vectors which contain the DNA-sequence, a promoter region or a chimeric nucleic molecule,] A vector comprising the nucleic acid sequence as set forth in Claim [1] 4, or fragments thereof.
- 11. (Amended) [Transgenic plants which contain the DNA-sequence, a promoter region or a chimeric-acid molecule,] A transgenic plant or constituent or propagation material thereof comprising the nucleic acid sequence as set forth in Claim [1] 4 [, as well as constituents of such plants and the propagation material thereof, such as protoplasts, plant cells, calli, seeds, tubers or cuttings, etc., as well as the offspring of such plants].

- 15. (Amended) [Plants,] A plant as set forth in Claim 11, [in which an ozone inducible gene expression of a gene in which said DNA] wherein said nucleic acid sequence does not naturally occur, and wherein ozone-inducible gene expression is conveyed [can take place].
- 18. (Amended) [Plants,] A plant as set forth in Claim 15, in which an ozone-inducible expression of reporter genes [can] occurs.
- 19. (Amended) [Dicotyle plants,] A plant as set forth in Claim 11, wherein the plant is a dicotyle plant [in particular useful plants, such as soya bean, rape, tomato, sugar beet, potato, cotton, tobacco, as well as ornamental plants or trees].
- 20. (Amended) [Monocotyle plants,] A plant as set forth in Claim 11, wherein the plant is a monocotyle plant [especially grain such as oat, wheat, rye, barley, rice, millet, or corn].
- 21. (Amended) [Transgenic plant cells, including protoplasts, which contain the DNA-] A transgenic plant cell comprising the nucleic acid sequence [, a promotor region or a chimeric nucleic-acid molecule,] as set forth in Claim [1] 4.
- 23. (Amended) [Plant cells,] <u>A plant cell</u> as set forth in Claim 21, [in which an ozone-inducible gene expression of a gene in which said DNA] <u>wherein said nucleic acid</u> sequence does not naturally occur, <u>and wherein ozone-inducible gene expression</u> can take place
- 27. (Amended) [Methods] <u>A method</u> for the production of transgenic plants or plant cells [in which] <u>comprising</u>:

introducing into said plant or plant cell a nucleic acid molecule comprising the sequence as set forth in claim 4, or a fragment thereof,

wherein one or several genes, the expression of which is not naturally occurring or not substantially induced by ozone, are ozone inducible, due to the introduction of the <u>nucleic acid</u> molecule [DNA sequence, as set forth in Claim 1, or a fragment thereof].

- 28. (Amended) [Methods,] <u>A method</u> as set forth in Claim 27, [in which] <u>wherein the</u> one or several genes are catalase and/or superoxide-dismutase genes [are ozone-inducible].
- 33. (Amended) A method for producing ozone-inducible characteristics in transgenic plants or plant cells [by] comprising:

inserting [the DNA] a nucleic acid molecule comprising the sequence, as set forth in Claim [1] 4, or at least a fragment thereof, into [those] genes which are not naturally or not substantially inducible through ozone.

- 38. (NEW) The isolated nucleic acid sequence of Claim 4, wherein the naturally occurring or artificially introduced variations are deletions, insertions, substitutions, additions, recombinations, or a combination thereof.
- 39. (NEW) The transgenic plant or constituent or propagation material of Claim 11, wherein the transgenic plant constituent or propagation material is a protoplast, a plant cell, a callus, a seed, a tuber. a cutting, or an offspring.
- 40. (NEW) A dicotyle plant as set forth in Claim 19, wherein the plant is soya bean, rape, tomato, sugar beet, potato, cotton, tobacco, or ornamental plant or tree.
- 41. (NEW) A monocotyle plant as set forth in Claim 20, wherein the plant is grain.
- 42. (NEW) A plant as set forth in claim 41,
- 43. (New) A method as set forth in claim 27, wherein the transgenic plants are biomonitors for the quantitative and/or qualitative determination of ozone-concentrations.